Cell-free dna methylation profiling for diffuse large b-cell lymphoma subtyping via whole-genome enzymatic methyl sequencing

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Abstract

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous group of malignancies of the immune system, arising from mature B cells and representing the most common aggressive lymphoma. While advances in molecular classifications have improved the DLBCL subtyping based on genetic and transcriptomic features, its genome-wide DNA methylation patterns remain incompletely characterized. Traditional diagnostic methods rely on tissue biopsies, which are invasive and may not fully capture tumor heterogeneity. Cell-free DNA (cfDNA), released into the bloodstream by tumor and normal cells, provides a unique opportunity to study these epigenetic features non-invasively. By analyzing cfDNA methylation signals, we aim to characterize DLBCL subtypes and explore their potential as biomarkers for disease classification and risk-profiling.

In this study, we analyzed plasma cfDNA and formalin-fixed, paraffin-embedded (FFPE) lymphoma tissue samples from ten diffuse large B-cell lymphoma (DLBCL) patients enrolled in Nordic Lymphoma Group (NLG) Phase II trials LBC-05 and LBC-06, including five germinal center B-cell (GCB) DLBCL and five non-GCB DLBCL patients. cfDNA from three healthy individuals was analyzed as controls. Whole-genome enzymatic methyl sequencing (EM-seq) and analysis with DRAGEN and Bismark were performed to identify genomic variants and DNA methylation calls.

cfDNA was extracted with a median concentration of 40 ng/mL. EM-seq data demonstrated high sequencing quality, with an 82% alignment rate, a median coverage depth of 28×, and <1% non-CpG methylation in cfDNA, indicating minimal methylation bias. Principal component analysis (PCA) of methylation profiles revealed clear separation between DLBCL patients and healthy controls, while capturing subtype heterogeneity between the GCB and non-GCB DLBCLs. Differential methylation analysis defined subtype-specific methylation patterns and identified CpG sites associated with distinct subtypes. These findings have potential to contribute to the characterization of cfDNA methylation landscapes in DLBCL, supporting the development of clinically relevant biomarkers for precision oncology and targeted therapies.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.